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Is RLS Primarily a Disorder of Wakefulness or of Sleep?

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Abstract: ***Restless leg syndrome (RLS) is a common sensorimotor disorder characterized by an abnormal sensation mainly in the legs causing an intense urge to move them repeatedly. It is prevalent in nearly 10% of the population. The sensory symptoms occur at rest, are relieved by movement and are predominantly experienced at night. Some features of RLS manifest in the evenings in a restful awake state, leaving one to wonder whether it is also a disorder of wakefulness or a 24-hour disorder. From various studies, there appears to be two distinct mechanisms for RLS symptoms-a hyperdopaminergic state responsible for the sensory-motor symptoms and a hyper glutamatergic state causing enhanced arousal. From this perspective, RLS can be called a disorder both of wake and sleep. Newer revelations will definitely help us to treat RLS more effectively and improve the quality of life of these patients.***

Keywords: Restless leg syndrome, urge to move, sleep disturbance, circadian pattern.

1. Introduction

Restless leg syndrome (RLS) is a common sensorimotor disorder characterized by an abnormal sensation mainly in the legs causing an intense urge to move them repeatedly. The sensory symptoms occur at rest, are relieved by movement and are predominantly experienced at night. RLS was first mentioned by Thomas Willis in 1685 and was recognized by Karl Ekbom as a separate clinical disorder in 1960 –and therefore also known as 'Willis-Ekbom' disease.

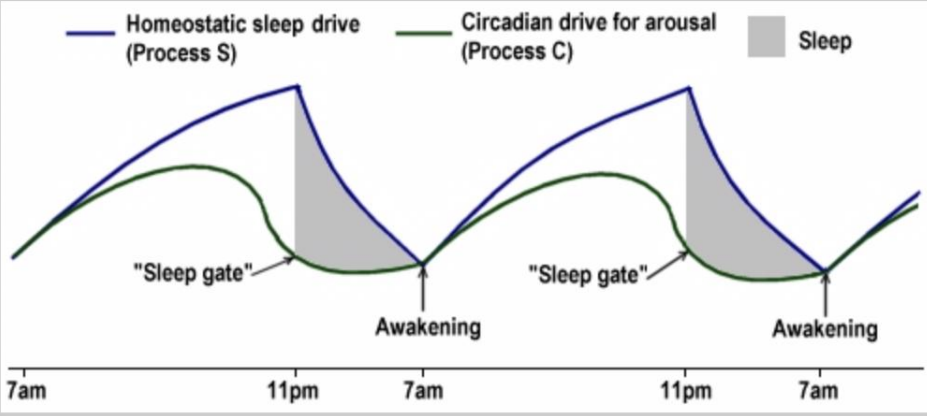
ICSD-3 Definition: The International classification of sleep disorders (ICSD 3) diagnostic criteria for RLS is as follows:

a) "An urge to move the legs, usually accompanied by or thought to be caused by uncomfortable and unpleasant sensation in the legs. These symptoms must:
   - Begin or worsen during periods of rest or inactivity such as lying down or sitting;
   - Be partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues, and
   - Occur exclusively or predominantly in the evening or night rather than during the day.

b) The above features are not solely accounted for as symptoms of another medical or a behavioral condition (e.g. leg cramps, positional discomfort, myalgia, venous stasis, leg edema, arthritis, habitual foot tapping.

c) The symptoms of RLS cause concern and distress, sleep disturbance or impairment in mental, physical, social, occupational, educational, behavioral or other important areas of functioning."1As per the ICSD-3 diagnostic criteria, RLS occurs predominantly in the latter half of the circadian cycle and causes significant sleep disruption.

Physiology of sleep and wake: The two-process model of sleep described by Alexander Borbely in 1982, proposes that the sleep cycle is regulated primarily by two mechanisms- 'process C' or circadian rhythm and 'process S' or homeostatic control which is the homeostatic sleep drive.[2]



Sleep-wake regulation: interaction between the homeostatic sleep drive (Process S) and the circadian drive for arousal (Process C) (image by Luke Mastin)

Figure 1

Circadian rhythms are biological oscillations with the time period of slightly more than 24- hours. The rhythm influences the physiological behavior of most organisms including the rest-activity cycle. Most of our physiological

functions follow a 24-hour rhythm which can be appreciated by measurement of core body temperature, levels of Cortisol, Growth hormone, Prolactin, Melatonin etc.

Sleep is also regulated by the homeostatic drive for sleep which increases with increasing periods of wakefulness. The sleep pressure correlates with accumulation of Adenosine in the brain extracellular space which is thought to be the main neurotransmitter inducing sleep. The wake promoting neurotransmitters include –

a) histamine, dopamine, noradrenaline and serotonin (monoaminergic system), and
b) acetylcholine (cholinergic system).

The orexin neurons present in the lateral hypothalamus excite the monoaminergic and cholinergic neurons in the wake state. Simultaneously, the monoaminergic neurons also inhibit the VLPO (ventro lateral preoptic nucleus of hypothalamus) or sleep switch resulting in wakefulness. As the day progresses, there is buildup of Adenosine. The circadian and homoeostatic drive then activate the sleep switch VLPO which in turn releases the inhibitory neurotransmitters GABA and Galanin. These neurotransmitters then act on the orexinergic neurons on the lateral hypothalamus. The monoaminergic and cholinergic neurons are then inhibited, resulting in sleep. [2]

RLS-a sleep versus wake disorder: In restless leg syndrome, sleep disturbance is usually present and was thought to be a result of 'akathisia', the unpleasant sensation in the legs or PLMS (periodic leg movements in sleep) which are seen in 80% of the patients of RLS. [3] Again, the clinical features of RLS consisting of the unpleasant sensory aspect as well as the motor disturbance of leg movements may lead one to wonder whether it is purely a neurological disorder or a movement disorder. The disturbing symptoms are present not only in sleep but usually between 4 PM and 4 AM. This circadian pattern is seen in mild and moderate cases. In severe stages, the disease may lose its circadian pattern. This leads to a dilemma whether it is indeed a disorder of sleep or also of wakefulness.

In clinical practice, insomnia is one of the main complaints with which a patient of RLS may present. [4] Difficulty in initiating sleep is one of the main features as described in some studies.[5]

Numerous studies have indicated the circadian pattern of RLS. Hening et al in 1999, correlated RLS peak intensity with the core body temperature drop.[6]Rozi Andretic and Jay Hirsh in 2000 demonstrated that dopamine receptor responsiveness has a circadian pattern.[7]Diego Gracia - Borreguero et al in 2004, demonstrated that the sensitivity of post-synaptic dopamine receptors increased at night in RLS patients at the level of tubero-infundibular-dopaminergic pathways.[8] It is well-documented that altered dopaminergic neurotransmission is responsible for akathisia and PLMS in RLS.[9] Earlier, benefits of L-dopa treatment on the sensorimotor features of RLS have been shown.

In 2004, Martin Michaud et aldemonstrated a correlation between the biological markers-subjective vigilance, core body temperature and salivary melatonin and the symptoms of RLS.[10]These studies point to circadian pattern of RLS, suggesting that it is a disorder of sleep rather than wakefulness.

In 2013, Allen et al investigated the role of the neurotransmitterglutamate in RLS and showed significant correlation between increased glutamate levels and subjective and 2 objective sleep disturbance.[11]They concluded that since hyperarousalis seen both during the day and night, RLS is a 24-hour disorder. Sergi Ferre et al in 2018 suggested that the hyperarousal state in RLS could be due to a hypoadenosinergic state induced by Brain Irondeficiency (BID) which causes down regulation of A1 receptors.[12] Thus, adenosine which normally would activate 'sleep switch' is deficient. Down regulation of A1 receptors would cause a disruption of the adenosine-dopamine-glutamate balance resulting in PLMS as well as sleep disruption. This suggests that sleep disturbance in RLS has a definite aetiology and is not merely due to the sensor motor symptoms of the disorder.

The following section of the essay will focus into the details of the various studies some of which provide evidence in favor of RLS as a sleep disorder, while a fewopine on the contrary.

Circadian Pattern of RLS:

A study was done by Claudia Trenkwalder et al in 1999, to determine whether PLMS and the akathisia of RLS are regulated by an independent circadian factor.[13] They examined eight RLS patients with severe RLS symptoms for three consecutive nights and days using PSG recording for first two nights, followed by a sleep deprived third night and the following day after sleep deprivation. Their study demonstrated that PLMS and akathisia both worsened at night with a peak intensity between 12 midnight and 1 AM and a minimum between 9 AM and 11 AM. They also measured core body temperature continuously using a rectal thermometer. Comparing the frequency of PLMS with CBT graphs, they found that the highest PLMS occurred on the falling phase of the temperature curve, with the peak number of PLMS occurring just prior to temperature nadir. Sensory complaints were maximum between 11 PM and 12:30 AM and lowest between 9 AM and 10:30 AM. They concluded that sensory symptoms and PLMS of RLS were worse at night, not only because the patient were sitting or lying down (resting) but were regulated by an independent circadian factor.They also suggested that circadian pattern of variation of catecholamines and other neurotransmitter secretion could certainly have a role to play in the pathogenesis of RLS. In their study, only core body temperature was correlated with the occurrence of the PLMS-and that too only in four patients. Moreover, the numbers of subjects recruited were only eight and there was no control group. However, this was one of the first few studies which suggested involvement of a definite circadian factor in the pathophysiology of RLS.

Hening et al in 1999, in a similar study concluded that a circadian factor modulates the RLS intensity as its peak correlates with the falling phase of the CBT. Nine patients

with clinically severe RLS were monitored with continuous ambulatory activity and CBT recording. Modified suggested immobilization tests (mSITS) were performed every three hours when subjects were awake. Subjective discomfort was also measured every 15 minutes using SITs. Motor activity was assessed through activity monitoring. Results showed sensory and motor symptoms were least in the morning and highest shortly after midnight. They found that this nadir in the morning occurred even after the night following sleep deprivation. This finding was similar to the previous study by Trenkwalder. It was also suggested in this study that circulating levels of dopamine, iron and ferritin which have a circadian rhythm and have been used for the treatment of RLS symptoms may have a role in the pathogenesis of RLS. Drawbacks of the study were that no controls were taken, few study subjects and the only circadian factor analyzed was CBT.

In a study on drosophila in 2000 by Rozi Andretic and Jay Hirsh, circadian control of dopamine receptor responsiveness was demonstrated.[7]They measured behavioral responses to a dopamine receptor agonist (Quinpirole) in decapitated flies. A circadian pattern to the dopamine receptor responsiveness was seen which showed highest responsiveness at night to Quinpirole. Dopamine is known to control motor behavior. In Parkinson's disease, there is a degeneration of the dopaminergic Nigro-striatal pathway that results in the resting tremors. As dopaminergic dysregulation is thought to be responsible for motor symptoms of RLS, the up-regulation of D2 receptor responsiveness at night may have a role to play in that exacerbation of symptoms at night and during sleep.

Another study by Diego Gracia-Borreguero et al in 2004, investigated the circadian variation in dopaminergic function in RLS patients.[8] The subjects were divided into two groups (12 RLS and 12 healthy controls) and given Levodopa with carbidopa orally at 11 AM and 11 PM. Pre-challenge and post challenge plasma values of growth hormone, prolactin and cortisol were examined using RIA (Radioimmunoassay) in both groups on both the occasions. No difference was found in prechallenge values among the two groups. Normally, administration of dopamine inhibits prolactin release and exacerbates growth hormone release. This phenomenon was found to be significantly enhanced in RLS patients as compared to healthy controls at night. Also, plasma prolactin levels correlated with PLMS as seen in PSG is recording. This study also concludes that there is enhanced responsiveness of post synaptic dopamine receptors at night in RLS patients.

In an interesting study by Martin Michaud et al in 2004, the circadian variations in RLS were compared with the circadian variations in subjective vigilance, CBT and salivary melatonin in seven patients of RLS as compared to age and sex-matched healthy controls.[10] The subjects were assessed using eight- hour PSG recording followed by a modified constant routine for the next 28 hours. These 28 hrs. were again divided into 14 episodes of two hours each. During each two-hour period sample for salivary melatonin was collected, SIT was done and the level of discomfort quantified on a visual analogue scale (VAS). Subjective vigilance was also assessed every hour on a VAS. CBT was measured constantly using a rectal thermistor. Salivary melatonin was analyzed through RIA. DLMO (Dim light melatonin onset) and DLM off was determined. Subjects' activity level was also measured using Actigraphy. The results of the study showed a clear circadian rhythm of leg discomfort and PLM in the RLS group, the acrophase occurring at 3 AM. While the control group also experienced some PLMS and discomfort this was not severe and the acrophase occurred between 4 to 6 AM. Subjective vigilance correlated negatively with PLM and leg discomfort as also CBT and legs symptoms. Strong relation was also seen between salivary melatonin and RLS symptoms. The changes in melatonin concentration preceded the increase of RLS symptoms by approximately two hours. Thus, they concluded melatonin may play a role in the pathophysiology of RLS, by decreasing the activity of central dopaminergic secretion. However, further studies were needed, to prove this hypothesis.

The above studies definitely point to the fact that RLS has a circadian regulation with a predominance in the latter half of the circadian cycle which also coincide with the 'rest' period of our 'rest-activity' cycle. Since wakefulness correlates with activity and rest with sleep, we cannot deny the fact that RLS is more of a disorder of sleep than of wakefulness.

Pathosiology of RLS:

a) Role of BID: BID has been regarded to play an important role in the pathophysiology of RLS since a long time. A study in 2006 by Christopher J Earley et al focused on decreased brain iron concentration in the early and late onset RLS using MRI.[14] The iron concentration was determined in ten brain regions using MRI in 22 early onset RLS subjects, 19 late onset RLS subjects and 39 controls. The mean iron-index was found to be significantly lower in the Substantia nigra of the early onset RLS patients as compared to the controls. Previous studies on CSF ferritin concentrations as well as MRI measurement of brain iron in RLS patients demonstrated a deficient iron state.[15, 16] Autopsy studies done previously also supported the hypothesis.[17, 18] As the findings were consistent with the previous MRI, CSF iron concentration and autopsy studies, it was concluded that BID existed in early onset RLS patients. However, the study could not show any difference in BID concentration between late onset RLS patients and controls. A review by Earley et al also supported the initial role of BID in the pathophysiology of RLS.[9]

b) Role of Dopamine: BID resulting in a hyperdopaminergic state in RLS has been suggested in many studies. In 2009, Connor et al studied substantia nigra and putamen from autopsy specimen of the brain of RLS patients and compared them with controls.[19] Also assays were done on a cathecholamine cell line and animal models of iron deficiency. RLS tissue showed a significant decrease in D2 receptors in the putamen and increase in tyrosine hydroxylase (TH) in the substantia nigra as compared to controls. Similar results were seen in the animal and cell models of iron deficiency. Their results suggested that iron deficiency leads to a hyperdopaminergic state in RLS patients.

c) Role of Glutamate: Allen et al in 2013, evaluated the role of an abnormal glutamatergic system producing hyperarousal in RLS. Using magnetic resonance spectroscopy imaging, a significant increase in thalamic concentration of glutamate in RLS patients was shown which correlated with time spent awake. Their findings suggested existence of a hyper glutamatergic state in RLS patients resulting in hyperarousal. A recent optogenetic study in rodents with BID was done by Yepes et al.[20] Using optogenetic micro dialysis approach, they measured concentration of glutamate released in response to optogenetic stimulation of cortico-striatal glutamatergic terminals. Results showed that lower frequency of optogenetic stimulation was required to release glutamate in BID rodents. They also showed that glutamatergic terminals in both BID induced RLS and control rodents responded well to local perfusion of pramipexole, ropinirole and gabapentin which counteracted optogenetically stimulated glutamate release. To summarize, the study showed that glutamate release in cortico-striatal region was responsible for symptoms of RLS. From the above studies it can be said that the pathophysiology RLS as far as symptomatology is concerned was due to a BID induced hyper dopaminergic state resulting in the akathisia and PLMS, and a hyper glutamatergic state resulting in hyperarousal.

d) Role of Adenosine: In a study by Quiroz et al in 2016, the role of adenosine in the aetiology of sleep disturbance in RLS was investigated.[31] The study was done on 36 male mice aged three weeks who were divided into three groups-one of which was the control. The control group received an iron diet, while the other two groups received iron deficient diets. There were maintained in a 12:12 hour light/dark cycle for 3 weeks. Blood samples were taken and total serum iron, unsaturated iron-binding capacity (UBIC) and ferritin were measured. They were then sacrificed and cortico-striatal brain samples were taken and adenosine and dopamine receptor concentration analyzed by western blot. BID was associated with down-regulation of A1 receptors. The BID induced mice showed a circadian sleep architecture similar to hyper arousal state of RLS. A1 receptor down-regulation in forebrain, cortex and hypothalamus was probably responsible for hyperarousal and sleep disturbance in RLS, they observed.

These studies and numerous others very well describe the pathophysiology of RLS. BID induces a hyper dopaminergic and hyper glutamatergic state, more pronounced in the evening and night. Normally, in the latter half of the day, adenosine level rises and suppresses the activity of dopamine and glutamate. In RLS, brain iron deficiency results in decreased adenosine levels and failure of suppression of the actions of dopamine and glutamate which is more pronounced at night and results in the symptoms of RLS. This clearly suggests that RLS is a disorder of sleep as there is a decreased homeostatic drive.

RLS - A 24-HR Disorder?

In an editorial in 2013, John W Winkelman raised the question whether RLS is indeed a sleep disorder, a movement disorder or a disorder of chronic pain.[3] Referring to the study by Allen et al, [11] he described the concept of hyperarousal in RLS resulting from a hyper glutamatergic state, similar to insomnia. The elevated glutamate level in the study by Allen et al is present both during the day and night. Therefore, he proposes RLS to be a 24-hour disorder which manifests as a nocturnal disorder because the hyperarousal state disturbs sleep at night. However, the simultaneous hypoadenosinergic state also caused by the BID is not considered in this article.

Initial studies of RLS mostly attributed PLMS to be the cause of disturbed sleep in RLS. A review by Thomas C. Wetter et al in 1997 describe RLS as a sensory motor disorder where the occurrence of PLMS disturbed sleep, similar to narcolepsy, sleep apnea syndrome or the REM-sleep behavior disorder.[21] However, later studies have shown that hyper arousals mostly precede that onset of PLMS in at least 40% of patients of RLS.[22]

Richard K Bogan in his article remarked that sleep disturbances in RLS maybe as a result of combined sensory symptoms of RLS and the presence of PLMS and PLMW.[23] As the disturbing sensory symptoms are perceived in the awake state, it can be well said that RLS cannot be considered purely as a sleep disorder.

A study by Jacques Mont Plaisir et al in 1998 compared two types of immobilization tests-suggested immobilization test (SIT) and forced immobilization test (FIT) in 16 RLS patients with equal number of controls.[24] More leg movements were seen in the RLS group as compared to controls during both the immobilization tests-but specially SIT. PLMS were also measured on two consecutive nights and a higher PLMS index was found in RLS patients. The SIT test they suggested has more advantages than PLMS as it does not require PSG and can be done throughout the day. Only 10 patients of RLS had positive PLMS out of the 16 patients studied. Thus, SIT was considered a better test as compared to PLMS. PSG findings showed a fragmented sleep with decreased sleep efficiency in RLS patients as compared to controls. The study concludes that RLS is a disorder of wakefulness as well as sleep. The increased sensitivity and specificity of SITs as compared to the PLMS in this study suggests that RLS indeed manifests during wakefulness.

Sleep in RLS With Comorbid Conditions

a) **RLS in Multiple Sclerosis (MS) patients:** In 2008, Moreira et al evaluated sleep disturbance in 44 patients of MS, 12 with RLS and 32 without RLS.[25] The study showed that RLS is common in MS and related to poor sleep quality, increased sleep latency, decreased sleep duration and efficiency. Also, RLS patients showed significantly increased fatigue as compared to non-RLS MS patients. Thus, RLS has a negative impact on sleep and therefore cognitive functions and quality of life are affected. Another study by M. Khatooni et al in 2017, showed that quite a significant number of MS patients suffered from RLS which also led to sleep disturbance.[26]

b) **RLS in ESRD patients:** In 2015, F. Chavoshi et al investigated the prevalence of sleep disturbance in RLS patients with ESRD who were undergoing hemodialysis.[27] They found that 31.7% of patients on hemodialysis out of 397 had RLS. These patients had

significantly more sleep disturbances such as insomnia, excessive daytime sleepiness and poor sleep quality as compared to those without RLS.

c) **RLS in DM2 patients:** R. P. Skomro et al in 2001, studied the prevalence and characteristics of sleep complaints in diabetic patients with RLS.[28] They concluded that sleep complaints are definitely more in adult diabetic patients with RLS.

2. Conclusion

Restless leg syndrome is a nocturnal neurological disorder which manifests with sensory motor symptoms and significant sleep disturbance. It is prevalent in nearly 10% of the population. In a study on the role of gene BTBD9 in the pathogenesis of RLS, it was seen that BTBD9-knockout mice showed significant increase in activity levels during the light phase but not during the dark phase showing they had significant rest phase activity .[29]These mice were seen to have circadian rhythm dependent hyperactivity as seen in RLS patients. Many studies have proved that sleep disturbance occurs and often is the most troublesome symptom for which patient of RLS seeks treatment. Some features of RLS manifest in the evenings in a restful awake state, leaving one to wonder whether it is also a disorder of wakefulness or a 24-hour disorder. Several studies have shown that a hyper glutamatergic state occurs throughout the 24-hour period in RLS resulting in hyperarousal both at night as well as during the day. In fact, RLS patients hardly demonstrate excessive daytime sleepiness as would be expected.

In their study, Shangru Lyu et al have shown that genetic alterations produce BID which causes functional and morphological alterations of the cerebral cortex and cortico-striatal-thalamic-cortical circuits. BID in RLS results from dysregulated iron transport across
the blood brain barrier (BBB). As already discussed, BID causes a presynaptic hyperdopaminergic state with increased synthesis and release of dopamine.

In 2013, Allen et al demonstrated and increase in basal glutamate levels in the thalamus of RLS patients.[11] A number on other studies also support this fact. Thus, there appears to be two distinct mechanisms for RLS symptoms-a hyperdopaminergic state responsible for the sensory-motor symptoms and a hyperglutamatergic state causing enhanced arousal. From this perspective, RLS can be called a disorder both of wake and sleep. Ferre et al in 2013, showed that a third state i.e. a hypoadenosinergic state maybe responsible for controlling the other states.[12]Adenosine inhibits presynaptic glutamate transmission through A1 receptors. The Adenosine receptors A1 and A2 also form heteromeric complexes with dopamine D1 and D2 receptors. These complexes normally inhibit dopamine signals by adenosine. Adenosine is the main neurotransmitter or sleep producing substance regulating the homeostatic component of sleep. As the day progresses, adenosine levels rise and promote sleep by acting on VLPO and inhibiting the ascending arousal system.

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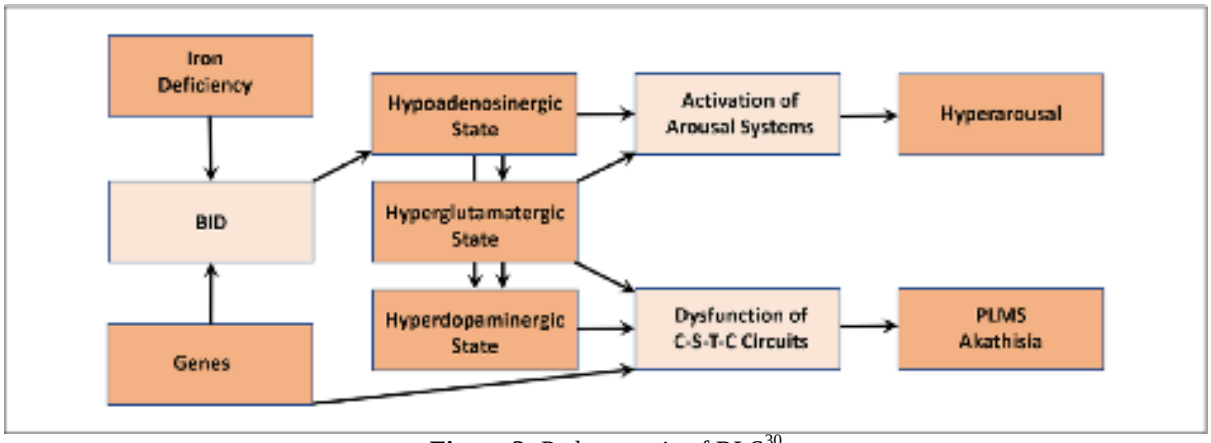

Figure 2: Pathogenesis of RLS[30]

Brain iron deficiency can occur from genetic predisposition and environmental factors. BID leads to a hypoadenosinergic state which fails to disinhibit the glutamatergic and dopaminergic pathways. This results in hyper glutamatergic and hyper dopaminergic states. The hypoadenosinergic and hyper glutamatergic states result in hyperarousal, while increased dopamine is responsible for the PLMS and akathisia.

To conclude, the evidence gathered from numerous studies point to the circadian pattern of RLS. Since we associate sleep with a fall in CBT and a rise in melatonin-RLS symptoms correlating with these definitely point to the fact that it is indeed a disorder of sleep rather than of wakefulness. The present revelations of the pathogenesis of RLS being due to the hypoadenosinergic state also relate to the fact that it is disorder of the homoeostatic mechanism of sleep. Sleep is a fundamental need four nervous regulation. Lack of homeostatic drive for sleep as in RLS leads to sleep disturbance, reduced cognitive functioning and impaired quality of life. The new revelations will definitely help us to treat RLS more effectively and improve the quality of life of these patients.

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